Hatch D
(1970) *Annals of Occupational Hygiene* **13**, 25
Hickish D E & Knight K L
(1970) *Annals of Occupational Hygiene* **13**, 17
Hilscher W, Sethi S, Friedrichs K H & Pott F
(1970) *Naturwissenschaften* **57**, 356
Holt P F, Mills J & Young D K
(1964) *Journal of Pathology and Bacteriology* **87**, 15
Lynch J R
(1968) *Journal of the Air Pollution Control Association* **18**, 824
Mizutani Y, Obara H & Nakajima K
(1973) *Wear* **23**, 387
Nicholson W J, Rohl A N & Ferraud E F
(1971) In: Proceedings of the Second International Clean Air Congress. Ed. H M England & W T Berry. Academic Press, New York; pp 136–139
Pott F, Huth F & Friedrichs K H
(1972) *Zentralblatt für Bakteriologie*, Abt. 1, Originale, **155**, 463
Rohl A N, Langer A M, Wolff M S & Weisman I
(1976) *Environmental Research* **12**, 110
Wagner J C, Berry G & Timbrell V
(1973) *British Journal of Cancer* **28**, 173

Neurotoxic Properties of Certain Aliphatic Hexacarbons

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During the summer of 1973 an outbreak of peripheral neuropathy developed among a large group of employees of a fabric manufacturing plant in Ohio, USA (Billmaier *et al.* 1974). The disease was characterized by distal weakness and sensory loss symmetrically in both the hands and the feet (Allen *et al.* 1975). The most severely affected individuals worked in the printing department where colouring inks, dissolved in volatile solvents, were applied to the surfaces of plastic coated fabrics. For several years the printing process required the use of a 9:1 solvent mixture containing methylethylketone (MEK) and methylisobutylketone (MIBK). Methyl-*n*-butylketone (MBK) was gradually introduced in the summer of 1972 to replace the MIBK. Methyl-*n*-butylketone was in its maximal use by December 1972 and the first case of peripheral neuropathy occurred shortly thereafter. The recent introduction of MBK into the printing department of the plant, coupled with other isolated outbreaks of neuropathy in individuals chronically exposed to MBK, suggested that this compound had neurotoxic properties. MBK produced peripheral neuropathy in several species of experimental animal (Duckett *et al.* 1974, Mendell *et al.* 1974, Spencer, Schaumburg, Raleigh & Terhaar 1975). These studies demonstrated that prolonged intoxication by inhalation or subcutaneous injection caused the insidious development of symmetrical weakness first in the hindlimbs and later in the forelimbs. The first signs of peripheral neuropathy developed after 4 to 12 weeks of continuous inhalation of 200–600 parts/10^6 of MBK, and after 12 to 16 weeks of intermittent inhalation of 1300 parts/10^6 of MBK. The onset of MBK neuropathy was associated with a reduction in the sciatic nerve conduction velocity (Mendell *et al.* 1974), an indication of nerve damage also found in rats and monkeys inhaling 1000 or 100 parts/10^6 MBK intermittently for periods of 3 and 8 months respectively (Johnson 1975), the figure of 100 parts/10^6 being the recommended Threshold Limit Value in the United States. Recent studies have suggested that concurrent exposure to MEK and MBK will produce neuropathy more rapidly than in animals exposed to MBK alone (Saida *et al.* 1976). Methylethylketone alone or MBK alone produce no neurotoxic effects (Spencer & Schaumburg 1976).

The purpose of the present paper is to emphasize the importance of chronic testing of potentially neurotoxic compounds in experimental animals, to describe the range of hexacarbon compounds which have been identified as neurotoxic agents and, finally, to characterize and illustrate the pathological basis for the onset of the nervous system disease. A total of seven hexacarbon compounds have been tested in this study (Table 1).

Table 1
Hexacarbon compounds tested

(1)	*n*-hexane $CH_3CH_2CH_2CH_2CH_2CH_3$
(2)	methyl-*n*-butylketone $CH_3COCH_2CH_2CH_2CH_3$
(3)	2,5-hexanedione $CH_3COCH_2CH_2COCH_3$
(4)	2,5-hexanediol $CH_3CHOH(CH_2)_2CHOHCH_3$
(5)	2,4-hexanedione $CH_3COCH_2COCH_2CH_3$
(6)	2,3-hexanedione $CH_3COCOCH_2CH_2CH_3$
(7)	1,6-hexanediol $HOCH_2CH_2CH_2CH_2CH_2CH_2OH$

The first compound, *n*-hexane, an important solvent and a minor component of petrol, was indicted in several reports as a possible neurotoxic agent (Herskowitz *et al.* 1971, Korobkin *et al.* 1975). Rats were exposed continuously to atmospheric levels of 400 to 600 parts/10^6 of *n*-hexane for up to five months, 500 parts/10^6 being the US Threshold Limit Value for *n*-hexane (*see also* Schaumburg & Spencer 1976). The second compound, methyl-*n*-butylketone, the solvent implicated in the outbreak of neuropathy, was administered to cats by subcutaneous injection of 150 mg/kg twice daily for periods up to six months (*see also* Spencer & Schaumburg 1976). The third compound, 2,5-

hexanedione, an intermediate in the synthesis of perfume ingredients, a tanning agent and a gasoline additive, and the remaining four compounds, 2,5-hexanediol, 2,4-hexanedione, 2,3-hexanedione and 1,6-hexanediol, were administered to rats in their drinking water as 0.5% solutions *ad libitum* for periods up to three months.

Four of the compounds proved to be neurotoxic (Table 1). The neurotoxic compounds each produced an identical type of slowly-developing, symmetrical peripheral neuropathy analogous to that seen in workers exposed to MBK. Animals first developed a waddling gait after several weeks of exposure, later were unable to extend their hindlimbs and eventually developed profound hindlimb weakness with footdrop. Severely affected animals also displayed weakness of the upper extremities.

All four neurotoxic compounds produced a uniform sequence and pattern of nerve fibre pathology, the degree of which was in direct proportion both to the level and duration of the intoxication. Nerve fibre degeneration was distributed widely in the nervous system. In the peripheral nervous system, nerve fibre degeneration was located in the distal regions of motor and sensory fibres located in the hindlimbs and to a lesser extent in the forelimbs. The distribution of peripheral nerve fibre pathology was studied in the rat by sampling branches of the sciatic/tibial/plantar nerve complex in the hindfeet. One of the most vulnerable parts of the peripheral nervous system appeared to be the tibial nerve branches supplying the calf muscles. Nerve fibres in these branches commenced degeneration before much longer fibres supplying muscles and sensory structures in the feet. With time, degeneration slowly ascended the affected nerve tracts and gradually involved more proximal parts of the sciatic nerve. Changes were also found in the spinal cord, medulla oblongata and cerebellum, and were contemporaneous with the commencement of peripheral nerve fibre degeneration. At these sites, nerve fibre degeneration began in the distal regions of long, ascending and descending spinal cord tracts and, with time, gradually moved along the affected nerve fibre pathways.

The pattern of peripheral and central nerve fibre degeneration was distinctive and was characterized by the early development within the distal portions of affected fibres of multifocal swellings of the axons (Figs 1, 2). The axonal swellings were also associated with localized changes in the myelin sheath apparently caused by slippage away from swollen regions of the axon. Affected portions of fibres underwent a complex series of pathological changes described elsewhere (Spencer & Schaumburg 1977*a*,*b*) which culminated in complete breakdown.

In summary, the present study revealed that animals chronically intoxicated with these neurotoxic hexacarbons slowly developed a peripheral neuropathy and displayed concurrent distal nerve fibre degeneration in the peripheral nervous system, and in ascending and descending spinal cord tracts of the central nervous system. The distribution and spatial-temporal evolution of this nervous system damage indicates that these compounds produce a primary axonal degeneration which first affects the distal regions of vulnerable nerve tracts and later progresses proximally. This type of peripheral and central nerve fibre degeneration, designated central-peripheral distal axonopathy, only develops after prolonged exposure to these compounds and cannot be reproduced using the acute and subacute exposure protocols usually adopted for the assessment of solvent toxicity.

The causation of nerve damage produced by the neurotoxic hexacarbons is totally unknown, but metabolic studies conducted by DiVincenzo *et al.* (1976) have provided some pointers. These investigators have suggested that methyl-*n*-butylketone

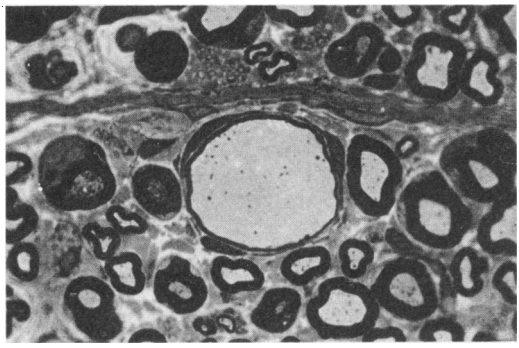

Fig 1 *Giant axonal swelling of a peripheral myelinated fibre (centre) seen in a cross-section of a sciatic nerve from a rat with 2,5-hexanedione neuropathy.* ×850

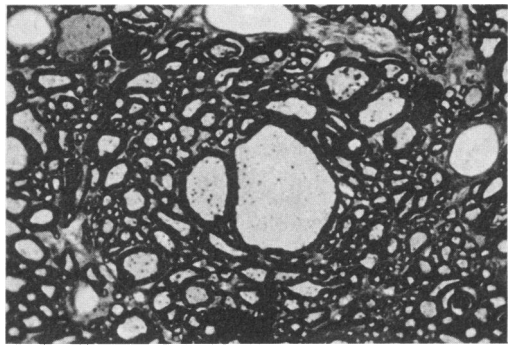

Fig 2 *Giant axonal swelling of a central myelinated fibre (centre) seen in a cross-section of the medulla oblongata from a rat with methyl-*n*-butylketone neuropathy.* ×850

is metabolized *in vivo* either to 2-hexanol and then to 2,5-hexanediol, or to 5-hydroxy-2-hexanone and then to 2,5-hexanedione. The studies reported here demonstrate that *n*-hexane, MBK, 2,5-hexanediol and 2,5-hexanedione are all neurotoxic agents. 2,5-hexanedione is of special interest since DiVincenzo and coworkers have demonstrated that this is the principal persistent metabolite of MBK. Furthermore, 2,5-hexanedione is able to exert its neurotoxic effect *in vitro*, presumably without further metabolism (Spencer, Peterson & Schaumburg 1975, Spencer, Schaumburg, Raleigh & Terhaar 1975). It seems likely, therefore, that 2,5-hexanedione is the primary neurotoxin of the neurotoxic hexacarbons. Whether the neurotoxic property of this compound is related to the symmetry of the molecule or to the delta spacing of the carbonyl groups, and where the substance acts in the nervous system, are subjects of current investigation in our laboratory.

REFERENCES

Allen N, Mendell J R, Billmaier D J, Fontaine R E & O'Neill J
(1975) *Archives of Neurology* **32**, 209

Billmaier D J, Yee H T & Allen N
(1974) *Journal of Occupational Medicine* **16**, 665

DiVincenzo G D, Kaplan C J & Dedinas J
(1976) *Toxicology and Applied Pharmacology* (in press)

Duckett S, Williams N & Frances S
(1974) *Experientia* **30**, 1283

Herskowitz A, Ishii N & Schaumburg H
(1971) *New England Journal of Medicine* **285**, 82

Johnson B
(1975) 5th Annual Meeting of Society for Neuroscience, New York

Korobkin R, Asbury A K, Sumner A J & Nielsen S L
(1975) *Archives of Neurology* **32**, 158

Mendell J R, Saida K, Ganansia M F, Jackson D B, Weiss H, Gardier R W, Chrisman C, Allen N, Couri D, O'Neill J, Maska B & Hetland L
(1974) *Science* **185**, 787

Saida K, Mendell J R & Weiss H S
(1976) *Journal of Neuropathology and Experimental Neurology* **35**, 207

Schaumburg H H & Spencer P S
(1976) *Brain* **99**, 183

Spencer P S, Peterson E R & Schaumburg H H
(1975) *Neuroscience Abstracts* **1**, 703. Society for Neuroscience, Bethesda, Md.

Spencer P S & Schaumburg H H
(1976) *Toxicology and Applied Pharmacology* **37**, 301

Spencer P S & Schaumburg H H
(1977*a*) *Journal of Neuropathology and Experimental Neurology* (in press)

Spencer P S & Schaumburg H H
(1977*b*) *Journal of Neuropathology and Experimental Neurology* (in press)

Spencer P S, Schaumburg H H, Raleigh R L & Terhaar C J
(1975) *Archives of Neurology* **32**, 219

The Elusive Marrow Toxin – Neutropenia of West Indians

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The paint sprayers in a car assembly factory recently requested routine health examinations. This followed training sessions which had been given by the medical officer to shop stewards on the Health and Safety at Work Act in general and concern about noise, lead and solvents in particular. Questionnaires and audiometry were completed for 168 primer and enamel sprayers before they started their shift. Chest X-rays, urinalysis, blood lead and blood count were also carried out. An unexpected finding among the very first tests was an appreciable number of blood counts showing an absolute neutropenia, but with essentially normal eosinophil, lymphocyte and platelet count. No increase in lead was found in their blood, which was checked by atomic absorption spectrometry because of the use of lead silicochromate in some enamel paints.

These neutropenias demanded investigation, and meetings with top management and technicians were called to institute a search for what seemed the likeliest cause, i.e. benzene. The research and development laboratory worked long hours analysing every paint, solvent, thinner, boothcoat – in all 53 materials – but no appreciable benzene content was found. At the same time all suppliers were asked by telex for up-to-date analysis of paints and thinners. The highest admitted and confirmed on gas chromatograph analysis was 0.7%, which is quite acceptable, 1.0% being the internationally agreed limit (Truhaut 1968). Many of the paints contain toluene and xylene. Xylene can reduce the platelet count (Forde 1973), but toluene if free of benzene is generally held not to cause neutropenia (Browning 1965, Hunter 1975).

Still in pursuit of benzene our hygiene specialists analysed the air of the paintspray booths and paint mix shop, but nothing abnormal was found, except that the concentration of xylene was fairly close to the toxic limit value (100 parts/10^6) in the paint mix room. At this stage urine analysis for phenols was introduced and these were found not to be raised, even among operators whose blood showed definite leukopenia. The blood counts of paint mixers were also checked, because they are much more exposed to fumes from paint and thinners than are the sprayers. All of them had normal blood counts.

Other industrial causes of absolute neutropenia are not described in the literature. Drugs of various kind can be a cause, but not all our subjects were